IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	: Brader, *et al.*	)	
		)	
Serial No.	: 08/484,542 ✓	)	
		)	Group Art Unit:
Filed	: 7 June 1995	)	1813
		)	
For	: Stabilized Acylated Insulin	)	
	Formulations	)	Examiner:
		)	Prickril
Docket No.	: 10361.49950	)	

DECLARATION UNDER 37 C.F.R. 1.131

Assistant Commissioner for Patents

Washington, D. C.   20231

Sir:

I, Michael J. Beckage, declare that:

I am a co-inventor and an applicant in this application.

Attached exhibits 1, 2, and 3 are copies of pages from my notebook in which I recorded the preparation of a formulation comprising an aqueous solution of a fatty acid-acylated insulin and approximately 0.35 mole of zinc per mole of said fatty acid-acylated insulin, and having a pH of approximately 7.5. The fatty-acid acylated insulin was N^ϵ-Lys^{B29}-palmitoyl-human insulin, which is referred to as C16-insulin. The formulation also contained *m*-cresol, a phenolic compound, at a concentration of approximately 2.5 mg/mL. The dates have been redacted.

All the actions, events, and observations described in this declaration occurred in the United States, prior to 23 March 1995.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

3/10/97
Date

Michael J. Beckage

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Reformulation of C16 - Insulin

DBF40 - UV potency 8/6/93 = 92 %

Assume 90% for calculations

U100 insulin = 3.54 mg/ml

$$U100 \quad C16 = \frac{6046}{5808} \times 3.54 = 1.041 \times 3.54 = 3.6435 \text{ mg/ml}$$

$$\frac{3.6435}{.9 \text{ mg Prot/mg. solid}} = 4.05 \text{ mg solids/ml}$$

dissolve in 60% final volume = 6.75 mg/ml

236.25 mg in 35 ml = 6.75 mg/ml

Stock diluent

Glycerol want 16 mg/ml   26.67 mg/ml in stock

6.69 grm in 250 ml .01N HCl = 26.76 mg/ml

Lot B1187   16.056 mg/ml after dilution

m-cresol density = 1.035 grms/ml

want final conc = 2.5 mg/ml

$$\text{Stock conc} = \frac{2.5}{.6} = 4.167 \text{ mg/ml} \times 35 = 145.8 \text{ mg}$$

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$$\frac{145.8 \, mg}{1.035 \, mg/ml} = \frac{140.86}{140.09} = 141 \, \lambda \; added$$

B64022 QA 168E

Material divided in 1/2

15 ml of protein stock to be diluted to 25 ml

Want .35 mole ratio of Zn to C16

3.6435 mg/ml C16 final conc x 25 ml = 91 mg

$$\frac{91}{6046} = .01505 \, mMoles$$

Want .01505 x .35 = 5.2679 x 10^{-3} mMoles

ZnO = 81.38 mg/mMole

5.2679 x 81.38 = .428 mg Zn $\frac{.428}{25} = .01712 \, mg/ml$

ZnO Stock
Y07223

$$\frac{11}{1.4 ml \; 1M HCl} = 7.857 \, mg/ml + 1.4 ml \; H_2O = 3.93 \, mg/m.$$

$$\frac{.428 \, mg \; ZnO}{3.93 \, mg/ml} = .1089 \, ml = 109 \, \lambda \; added$$

Recorded by: M. Beckman   Date _____   Verified by: _____   Date _____

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"Zn containing" formulations

ZB	200λ	1N	NaOH	turbid	
	25λ	1N	NaOH	turbid	
pink	25λ	1N	NaOH	turbid	pH 5.1
	100λ	1N	NaOH	clear	pH 7.88

QS to 25 ml divide in two parts

5λ	1N	NaOH	pH	8.05
5λ	.1N	HCl	pH	8.04
10λ	.1N	HCl	pH	8.0

ZA   start with second ½   pH 7.88

grey	10λ	1N	HCl	pH 7.42	clear
	5λ	.01N	NaOH	pH 7.49	

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Recorded by: _____   Date ____   Verified by: _____   Date ____